

Symbiotic Response of *Phyllanthus emblica* L. Vars. Wild and Chakaiya to Different Arbuscular Mycorrhizal Fungi

Mahesh B. Byatanal and H.C. Lakshman

Department of Botany, Microbiology Lab, Karnatak University Dharwad - 580 003, Karnataka, India

Abstract: Four different arbuscular mycorrhizal (AM) fungi were evaluated for their symbiotic response with *Phyllanthus emblica* L. (var. Wild and Chakaiya) under green house conditions. The AM fungi used were *Sclerocystis dussi*, *Acaulospora laevis*, *Gigaspora margarita* and *Glomus fasciculatum*. Seedlings were raised in polythene bags containing soil inoculated with isolates of different AM fungi. *Phyllanthus* seedlings raised in the presence of AM fungi generally had greater plant height, biomass, stem diameter, number of leaves and phosphorus content compared to uninoculated plants. They also had more mycorrhizal root colonization and spore numbers in the rhizospheric soil. Considering the various parameters of the plants, it was observed that *Glomus fasciculatum* is the best arbuscular mycorrhizal symbionts for *Phyllanthus emblica* L. (var. Wild and Chakaiya); compared to others in this experiment.

Key words: Arbuscular mycorrhiza % *Phyllanthus emblica* L. % Roots colonization % Plant morphogenesis

INTRODUCTION

Phyllanthus emblica L. is a medicinal plant with numerous medicinal properties. It is a branched tree which grows up to a height of 10-20 m. In the tropical region, it is supposed to be an evergreen tree but behaves as a deciduous tree due to complete defoliation of leaves. Every part of *Phyllanthus emblica* L. has medicinal use and is used for treating chronic dysentery, bronchitis, diabetes, fever, diarrhoea, jaundice, dyspepsia, cough, etc. Various plant parts are also used in toothache, anaemia, epilepsy, sores, pimples, fistula, gravel, cholera, sterility in women, anthrax [1].

The current day emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus, use of microbial inoculants play an important role in sustainable agriculture [2]. Arbuscular mycorrhizal fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and offer resistance to drought and salinity [3]. Though these fungi are not host specific, recent studies have clearly brought out host preference in arbuscular mycorrhizal fungi. Host preference has been reported in many forest tree species like *Casuarina equisetifolia* [4]; *Tectona grandis* [5]; *Garcinia indica* [6] and a few medicinal plant species like *Phyllanthus amarus* and *Withania somnifera*

[7] and *Coleus forskohlii* [8]. Hence, in the present investigation, it is envisaged the need for selecting efficient arbuscular mycorrhizal fungi for inoculating *Phyllanthus emblica* L. for its cultivation.

MATERIALS AND METHODS

Experimental Soil: The physico-chemical characteristics of the soil used for pot experiment were tested in soil testing laboratories at Jalavahini Management Private Limited, Dharwad district, India. The results are as follows: Soil was sandy loam with pH 8.1, Electric conductivity 320 uS/cm, Moisture 4.86%, Total organic Carbon 1.71, Nitrogen 0.08%, Phosphorus 4.52%, Potassium 7.94%, Magnesium 0.121%, Calcium 0.472%, Copper 0.03ppm, Zinc 3.86ppm, Manganese 0.97ppm, Iron 8.24ppm. The soil was steam sterilized for 1 hour on two consecutive days.

Arbuscular Mycorrhizal Inoculum Production: Arbuscular mycorrhizal fungal spores were isolated from the rhizosphere soil of species of *Phyllanthus emblica* L. (var. Wild and Chakaiya) collected from Botanical garden, Karnatak University and Gungarghatti nursery of Dharwad, Karnataka state, India. Arbuscular mycorrhizal fungal spores were isolated following the wet sieving and decanting method [9]. The spores were identified by using the manual written by Schenck *et al.* [10]. Genera of

Acaulospora, *Gigaspora*, *Glomus*, *Scutellospora* and *Sclerocystis* were isolated from 100 gram rhizosphere soil samples. Four species of arbuscular mycorrhizal fungi as *Sclerocystis dussi*, *Acaulospora laevis*, *Gigaspora margarita* and *Glomus fasciculatum* were dominant in this soil and were therefore selected for use in this experiment. Isolated spores were multiplied using Onion as host plants, for three months in culture pots measuring 15cm height, 30cm diameter pots were filled with autoclaved soil (121°C for 1h at 15 psi) from the same site. After three months onion was cut at ground level, the roots were chopped into small pieces and mixed with the soil from rhizosphere of host plants. This soil based inoculum was used for inoculation.

Soil Preparation and Seed Sowing: Experimental soil was collected from garden and stored in a greenhouse. 10 kg of sterilized soil was filled in each pot. Arbuscular mycorrhizal inoculum was added at the rate of 10g and seeds of *Phyllanthus emblica* L. (var. Wild and Chakaiya) were scarified in 5% H₂SO₄ for 10 min, washed with sterilized distilled, then sown in prepared pots. Germinated seedlings were raised in polythene bags containing soil inoculated individually with isolates of different AM fungi. Seedlings were selected for uniformity and one seedling per pot was planted.

Establishment of Test Plants and Greenhouse Experiments: The studies were conducted under greenhouse conditions with temperature ranges from 28-31°C. Arbuscular mycorrhizal fungal treatments were given on a layer below the germinated seedlings to the pot soil. Control (without mycorrhiza inoculation), 10g inoculum/10kg soil was given to each pot inoculated with *Sclerocystis dussi*, *Acaulospora laevis*, *Gigaspora margarita* and *Glomus fasciculatum*. The experiment was completely randomized with three replications per treatment of the two Wild and Chakaiya. All the pots are maintained under greenhouse conditions. To maintain moisture, pots were watered on every alternate day. A volume of 20 ml of Hogland solution was added to all the pots once every fifteen days.

Growth Parameters: Three seedlings per treatment were harvested with rhizosphere soil at 60, 120 and 180 days interval. Roots were washed thoroughly with tap water to remove adhering soil particles. Shoot and root fresh and dry weight, stem diameter, number of leaves, per cent of root colonization, spore number and phosphorus content in shoots were recorded. Shoot and root dry weight were also recorded after drying for 72h in a hot air oven at 80°C.

Mycorrhizal Status: Roots were processed using Phillips *et al.* [11], technique to study the per cent of root colonization. Arbuscular mycorrhizal fungal spores were isolated by wet sieving and decanting method [9].

Phosphorus Content: The phosphorus content in the shoots was determined by the vanado-molybdate phosphoric acid yellow color method outlined by Jackson [12].

Statistical Analyses: The data were statistically analyzed using Analysis of Variance (ANOVA) and the means were separated by Duncan's Multiple Range Test (DMRT) using SPSS 7.5 [13].

RESULTS

In general, all the recorded parameters showed gradual increase parallel to the increase in plant age as 60, 120 and 180 days of plant growth. Mycorrhizal inoculation resulted in a significant increase in plant height, biomass, stem diameter, number of leaves and phosphorus content of *Phyllanthus emblica* L. (var. Wild and Chakaiya). The highest plants were observed in both varieties of *Phyllanthus emblica* L. The highest plants were observed in inoculated with *Glomus fasciculatum* and differing significantly from other treatments and the least being in the uninoculated control plants (Table 1, 2 and Fig. 1, 2). The highest plant biomass was recorded in plants inoculated with *Glomus fasciculatum* followed by *Sclerocystis dussi*, *Acaulospora laevis* and *Gigaspora margarita*. Uninoculated control plants had significantly the least plant biomass compared to all other inoculated treatments (Table 1, 2 and Fig. 1, 2).

In both varieties of *Phyllanthus emblica* L. all the four inoculation treatments showed high shoot P content and differed significantly among each other. The highest shoot P content was observed in plants inoculated with *Glomus fasciculatum* and the least P content was observed in uninoculated plants (Table 1, 2 and Fig. 1, 2).

In this study highest mycorrhizal percent colonization was observed in plants treated with *Glomus fasciculatum* (Table 1, 2 and Fig. 1, 2), followed by other treatments. Highest number of mycorrhizal spores in the root zone soil was also observed in *Glomus fasciculatum* treated plants. Least number of spores occurred in the root zone of uninoculated plants (Table 1,2).

Table 1: Effect of soil inoculation with AM fungi on growth parameters, per cent of root colonization, spore number and phosphorus uptake in shoot of *Phyllanthus emblica* L. (Wild variety)

	SL(cm)	FWS(gm)	DWS(gm)	RL(cm)	FWR(gm)	DWR(gm)	STD(cm)	NL	PC(%)	SN	P (%)
60 DAYS											
CN	27.33d±0.33	1.55e±0.00	0.48b±0.00	17.90e±0.20	0.78e±0.00	0.11e±0.00	0.50e±0.00	09.33e±0.33	00.00e±0.00	00.00e±0.00	0.06e±0.00
SD	38.00b±0.57	7.67b±0.00	1.96a±0.00	37.10b±0.30	1.92b±0.00	0.63b±0.00	0.90b±0.00	17.66b±0.33	75.33b±0.33	158.66b±1.33	0.14b±0.00
AL	34.33c±0.33	2.94c±0.00	0.92b±0.00	34.23c±0.26	0.97c±0.00	0.17c±0.00	0.80c±0.00	12.33c±0.33	70.66c±0.33	152.00c±1.15	0.13c±0.00
GM	28.66d±0.33	2.43d±0.02	0.84b±0.00	30.06d±0.12	0.93d±0.00	0.13d±0.00	0.60d±0.00	11.00d±0.57	60.66d±0.66	127.66d±0.88	0.11d±0.00
GF	53.00a±0.57	8.68a±0.00	2.01a±0.66	46.66a±0.44	4.92a±0.00	1.27a±0.00	0.10a±0.00	19.66a±0.33	81.00a±0.57	172.33a±1.76	0.17a±0.00
120 DAYS											
CN	81.00e±0.57	22.83e±0.00	6.44e±0.00	47.00e±0.57	11.38e±0.00	2.40e±0.00	0.70e±0.00	38.66e±0.33	0.00e±0.00	0.00e±0.00	0.09e±0.00
SD	139.00b±0.57	45.93b±0.00	13.15b±0.00	82.66b±0.33	20.64b±0.00	4.98b±0.00	1.20b±0.00	77.66b±0.33	79.33b±0.33	176.33b±2.02	0.18b±0.00
AL	127.00c±0.57	36.94c±0.00	10.57c±0.00	77.00c±0.57	16.83c±0.00	4.21c±0.00	1.00c±0.00	68.00c±0.57	73.33c±0.33	164.66c±1.76	0.14c±0.00
GM	99.00d±0.33	32.64d±0.00	9.59d±0.00	65.33d±0.66	14.94d±0.00	3.83d±0.00	0.80d±0.00	64.66d±0.33	66.66d±0.33	135.00d±2.64	0.11d±0.00
GF	141.66a±0.88	72.34a±0.00	22.45a±0.00	89.00a±0.57	28.04a±0.00	7.99a±0.00	1.50a±0.00	82.66a±0.33	84.00a±0.57	183.00a±0.57	0.21a±0.00
180 DAYS											
CN	153.33e±0.33	54.26e±0.00	24.99e±0.00	81.00e±0.57	24.87e±0.00	13.81e±0.00	2.90e±0.00	72.00e±0.57	0.00e±0.00	0.00e±0.00	0.12e±0.00
SD	165.33b±0.66	84.52b±0.00	33.11b±0.00	112.33b±1.20	38.21b±0.00	18.54b±0.00	3.20b±0.00	100.00b±0.57	81.33b±0.33	189.66b±2.60	0.24b±0.00
AL	151.00c±0.57	73.56c±0.00	30.41c±0.00	97.00c±0.57	35.69c±0.00	18.50c±0.00	3.10c±0.00	96.66c±0.33	77.66c±0.88	180.33c±1.20	0.18c±0.00
GM	141.33d±0.88	64.03d±0.00	27.54d±0.00	86.66d±0.33	35.60d±0.00	15.88d±0.00	3.00d±0.00	75.66d±0.33	74.00d±0.57	155.66d±3.33	0.16d±0.00
GF	170.66a±0.33	108.80a±0.00	42.85a±0.00	120.33a±0.88	76.12a±0.00	35.78a±0.00	3.90a±0.00	121.66a±1.20	91.00a±0.57	216.66a±3.52	0.28a±0.00

SL-Shoot Length; FWS-Fresh Weight of Shoot; DWS-Dry Weight of Shoot; RL-Root Length; FWR-Fresh Weight of Root; DWR-Dry Weight of Root; STD-Stem Diameter; NL-Number of Leaves; PC-Per cent Colonization; SN-Spore Number; P-Phosphorus uptake in shoots.

CN-Control; SD-*Sclerocystis dussii*; AL-*Acaulospora laevis*; GM-*Gigaspora margarita*; GF-*Glomus fasciculatum*.

Means sharing a letter in columns are not significantly different according to Duncan's test P<0.05.

All the values are mean of three replicated±Standard error

Table 2: Effect of soil inoculation with AM fungi on Growth parameters, per cent of root colonization, spore number and phosphorus uptake In shoot of *Phyllanthus emblica* L. (Chakaiya variety)

	SL(cm)	FWS(gm)	DWS(gm)	RL(cm)	FWR(gm)	DWR(gm)	STD(cm)	NL	PC (%)	SN	P(%)
60 DAYS											
CN	13.33d±0.33	1.85e±0.00	0.66e±0.00	34.66d±0.33	0.01e±0.00	0.18e±0.00	1.00e±0.00	8.00c±0.57	0.00e±0.00	0.00d±0.00	0.05e±0.00
SD	22.66b±0.33	4.94b±0.00	1.76b±0.00	40.33b±0.33	2.02b±0.00	0.65b±0.00	1.40b±0.00	13.00a±0.57	86.66b±0.33	141.66b±1.76	0.16b±0.00
AL	18.66c±0.33	3.19c±0.00	1.45c±0.00	38.00c±0.57	1.97c±0.00	0.60c±0.00	1.30c±0.00	11.00b±0.57	74.00c±0.57	136.00b±1.00	0.14c±0.00
GM	13.66d±0.33	3.07d±0.00	0.99d±0.00	35.00d±0.57	1.27d±0.01	0.28d±0.00	1.20d±0.00	10.33b±0.33	65.00d±0.57	129.00c±1.73	0.11d±0.00
GF	28.33a±0.33	7.26a±0.00	2.36a±0.00	56.00a±0.57	3.82a±0.00	0.97a±0.00	1.50a±0.00	14.33a±0.33	96.33a±0.33	152.66a±3.48	0.19a±0.00
120 DAYS											
CN	17.33e±0.33	7.46e±0.00	2.61e±0.00	44.00e±0.57	10.58e±0.00	2.83e±0.00	1.70e±0.00	12.00d±0.57	0.00e±0.00	0.00e±0.00	0.07e±0.00
SD	28.33b±0.33	20.58b±0.00	7.71b±0.00	67.00b±0.57	27.38b±0.00	8.70b±0.00	2.10b±0.00	17.66b±0.33	90.00b±0.57	161.33b±1.20	0.19b±0.00
AL	26.33c±0.33	15.01c±0.00	5.74c±0.00	64.33c±0.33	21.68c±0.00	5.00c±0.00	2.00c±0.00	14.66c±0.33	77.00c±0.57	147.66c±1.45	0.16c±0.00
GM	22.00d±0.57	12.62d±0.00	4.33d±0.00	60.66d±0.66	13.89d±0.00	4.41d±0.00	1.90d±0.00	12.66d±0.33	67.66d±0.33	137.66d±1.33	0.13d±0.00
GF	40.00a±0.57	29.99a±0.00	10.24a±0.00	78.33a±0.33	32.25a±0.00	10.65a±0.00	2.60a±0.00	28.00a±0.57	96.00a±0.57	173.00a±6.08	0.24a±0.00
180 DAYS											
CN	22.00e±0.00	9.32e±0.00	4.26e±0.00	49.33e±0.33	11.21e±0.00	3.83e±0.00	2.20e±0.00	19.66d±0.33	0.00e±0.00	0.00d±0.00	0.12e±0.00
SD	30.33b±0.33	21.63b±0.00	8.88b±0.00	73.33b±0.33	31.32b±0.01	10.45b±0.00	2.90b±0.00	27.00b±0.57	91.00b±0.57	168.66b±0.33	0.26b±0.00
AL	28.00c±0.57	18.04c±0.00	7.35c±0.00	69.66c±0.33	23.54c±0.00	7.12c±0.00	2.80c±0.00	22.66c±0.33	78.66c±0.33	162.00b±4.72	0.19c±0.00
GM	25.00d±0.57	15.80d±0.00	6.63d±0.00	67.33d±0.88	14.62d±0.00	5.03d±0.00	2.50d±0.00	20.66d±0.88	73.33d±0.33	140.66c±1.20	0.16d±0.00
GF	51.00a±0.57	31.42a±0.00	16.09a±0.00	83.66a±0.33	34.06a±0.00	12.52a±0.00	3.10a±0.00	33.66a±0.33	98.00a±0.00	181.00a±1.52	0.32a±0.00

SL-Shoot Length; FWS-Fresh Weight of Shoot; DWS-Dry Weight of Shoot; RL-Root Length; FWR-Fresh Weight of Root; DWR-Dry Weight of Root; STD-Stem Diameter; NL-Number of Leaves; PC-Per cent Colonization; SN-Spore Number; P-Phosphorus uptake in shoots.

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Means sharing a letter in columns are not significantly different according to Duncan's test P<0.05.

All the values are mean of three replicated±Standard error

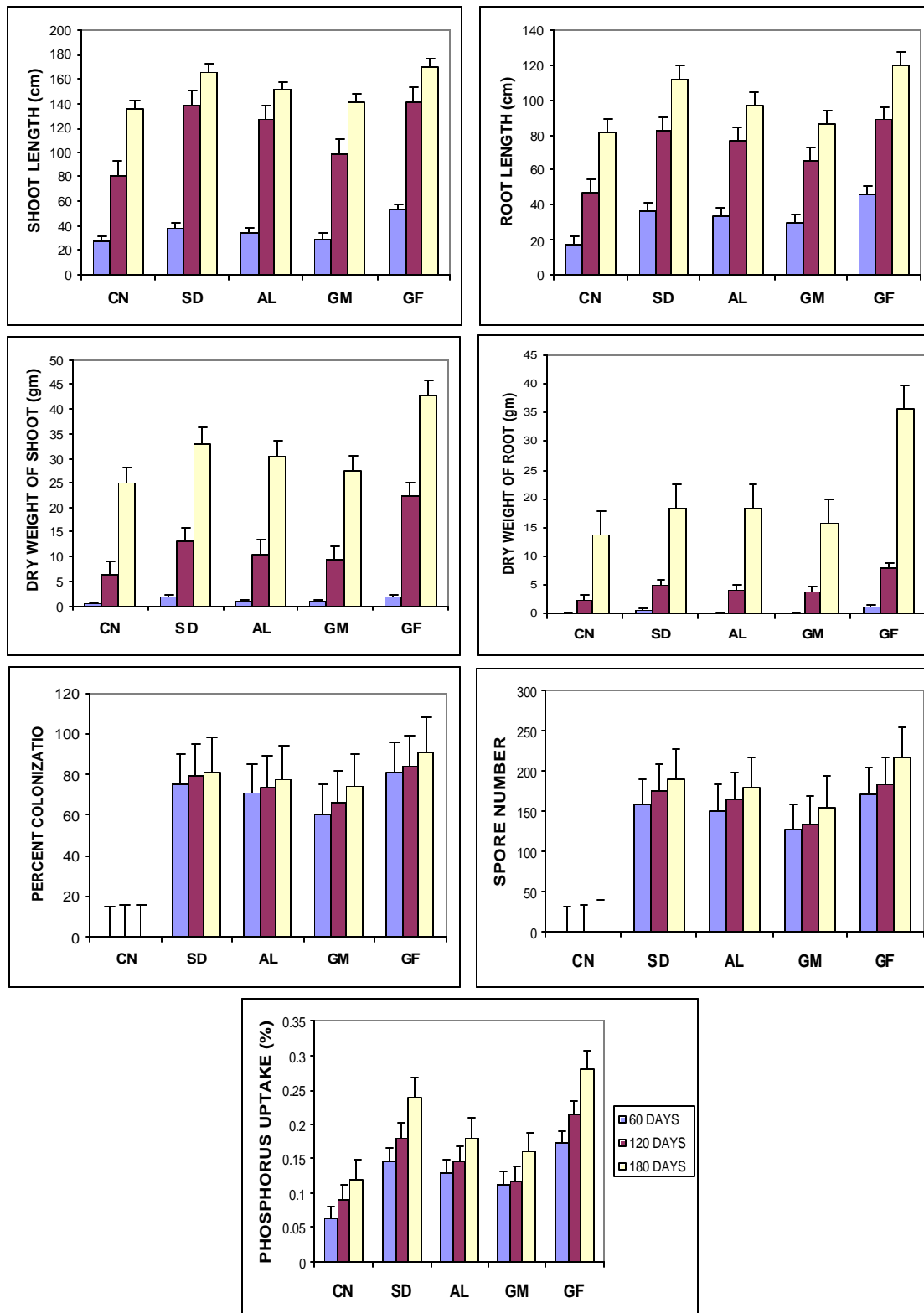


Fig. 1: Effect of AM fungi on growth parameters, per cent of root colonization, spore number and Phosphorus uptake of *Phyllanthus emblica* L. (Wild variety) 60,120 and 180 days under green house conditions. CN-Control; SD-*Sclerocystis dussi*; AL-*Acaulospora laevis*; GM-*Gigaspora margarita*; GF-*Glomus fasciculatum*.

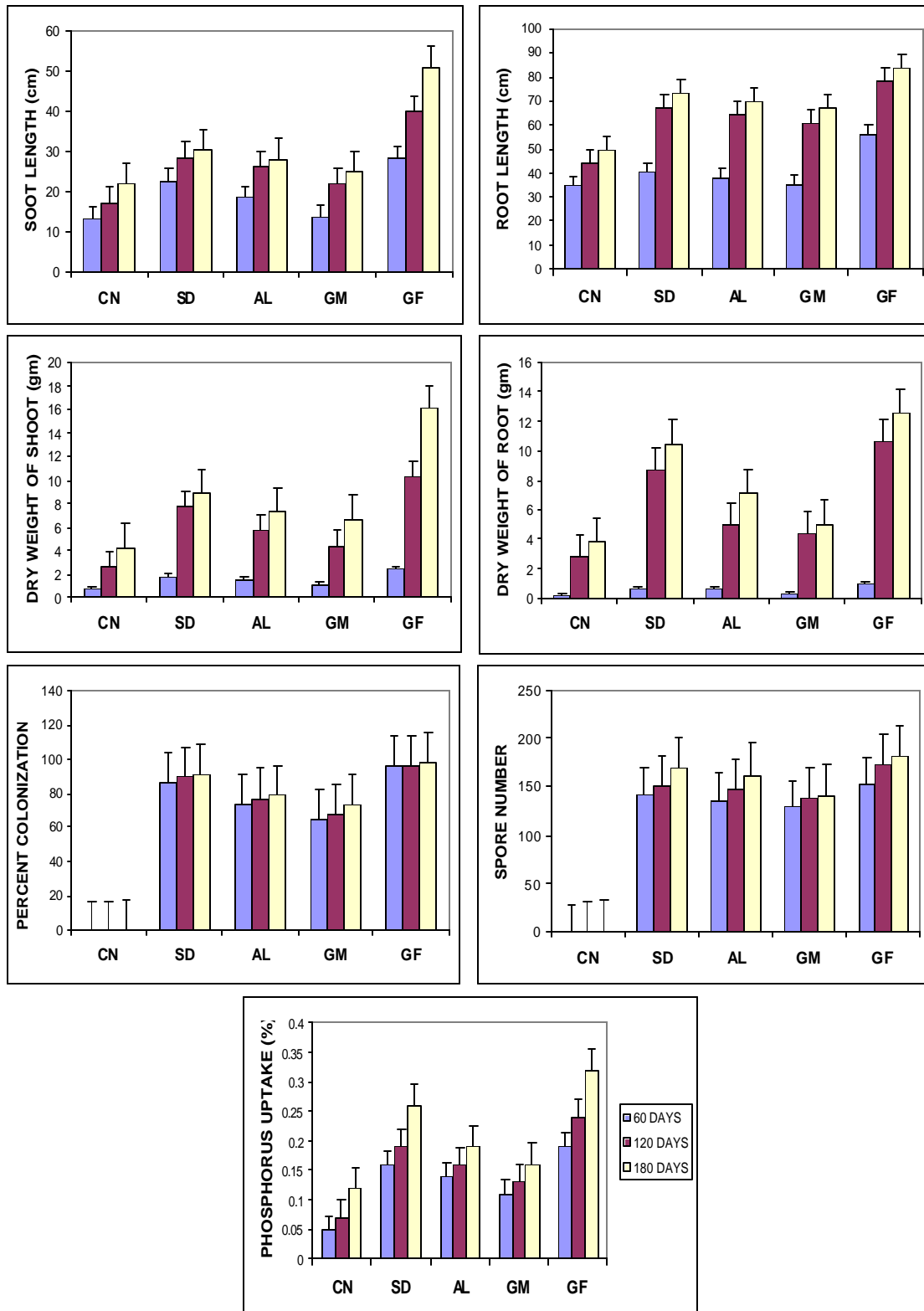


Fig. 2: Effect of AM fungi on growth parameters, per cent of root colonization, spore number and Phosphorus uptake of *Phyllanthus emblica* (Chaikaiya variety) 60,120 and 180 days under green house conditions. CN-Controlle; SD-*Sclerocystis dussii*; AL-*Acaulospora laevis*; GM-*Gigaspora margarita*; GF-*Glomus fasciculatum*.

DISCUSSION

Both varieties of *Phyllanthus emblica* L. plants Wild and Chakaiya varied in their response to inoculation with different mycorrhizal fungi. Most of the AM fungi resulted in significant increase in plant height, plant biomass, stem diameter, number of leaves and phosphorus content of *Phyllanthus emblica* L. tested varieties. Host preference among AM fungi has been reported by Gracy *et al.* [8] and McGraw and Schenk [14]. Hence, the need for inoculating different mycotrophic plants has been stressed [3, 15]. Improved plant height, because of AM fungal inoculation has been reported in other medicinal plants like coleus [16] and kalmegh [17]. The plant biomass is an important parameter for selecting a fungus for its symbiotic efficiency. Plant biomass (shoot + root) was enhanced due to *Glomus fasciculatum* inoculation compared with uninoculated plants. Increase in the plant biomass because of inoculation with AM fungi has been reported in aromatic plants like palmarosa [18], eucalyptus [19], bergamot mint [20] and sweet basil [21].

Increase of the plant growth due to AM fungal inoculation is mainly through improvement the uptake of diffusion limited nutrients such as P [8, 22-24]. AM fungi improving plant biomass were also good in increasing the P content of the host, significantly the highest being in plants inoculated with *Glomus fasciculatum*. Selected efficient fungi enhancing plant biomass and P uptake has been reported also in other plants by several workers [4, 25]. Such higher P content in AM fungal inoculated plants is attributed to higher influx of P into the plant system through AM fungi which explores the soil volume beyond P depletion zone [26-28]. The enhancement in growth and nutritional status was also related to mycorrhizal root colonization and spore numbers in the root zone soil. This upholds the observations made by earlier workers on other plants [8, 17].

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